

**CONFIDENTIAL**

TIRC Grant #74

Progress Report #2

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"Quantitative Analysis of Nucleoproteins in Tissues from  
Animals Subjected to Tobacco Smoke by Microspectrophotometry  
and Interference Microscopy Correlated with Cytological and  
Histological Studies"

From the time the grant was received until now (a period of 10 months), a special laboratory has been set up to carry out the studies as outlined in topic No. 5 of the application form.

Preliminary trials with the Essenberg smoking machine were not too satisfactory because the performance of the machine was erratic and could not be standardized. Therefore it was necessary to redesign this machine and improve it. A photograph of the modified machine is enclosed with a short description. We feel that this newly built machine which has been in operation for a few months is satisfactory because mice can be exposed to cigarette smoke under standardized and reproducible conditions.

Up to now, approximately 50 mice have been studied after exposure to cigarette smoke for periods varying from 1 to 60 days at varying intervals during each day and with varying numbers of cigarettes. The same number of mice were kept in the control chamber (see "L" in photograph). For each experiment, animals from both the experimental and control groups were killed at the same time and appropriate tissues were immediately removed for fixation.

Histological, cytological and microspectrophotometric studies have been made on the bronchial and lung tissues from these animals. Up to the present time, no significant microscopic differences have been found in these tissues between the groups exposed to cigarette smoke and the ones not exposed.

Extensive microspectrophotometric determinations of the deoxyribose nucleic acid (DNA) content have been made in more than 2000 cells from the bronchi and lungs of the smoked and non-smoked animals. Again, no significant differences have been found between the exposed and non-exposed animals. Both these groups show surprisingly constant diploid amounts of DNA in the cells from the bronchi and lungs. Although it is obviously too early to draw any conclusions on the basis of these data, it seems justified to say that up to the present, no hyperplasia has been observed in mice exposed to cigarette smoke under the conditions described above.

These DNA studies will be continued because it is felt that longer periods of exposure may perhaps result in some changes. An excellent baseline has now been established for comparison with the future long term experiments planned. To the best of our knowledge, no such data have been reported.

Similar studies have been started for dry mass and protein determinations. However, due to the great difficulty in elaborating proper technics for these special tissues, the data are still too scanty to permit even a preliminary analysis now.

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Description of the Smoking Machine used for the Experiments Described in the enclosed Progress Report.

This machine is made of plastic and was built at the Institute of Pathology, Western Reserve University. The disc which transports the cigarettes was built by Dr. J. Essenberg at the University of Chicago.

By means of the vacuum pump (A), air is withdrawn from the smoking chamber (E) at outlet (B) and control chamber (L) at outlet (C). These two chambers are separated by an airtight plastic partition (D).

Air passes from the outside through a Seitz filter (F) into the cigarette house (G), through a cigarette (H), into the smoking chamber (E) by means of an opening at (I) in front of a burning cigarette (H).

An electric clock (J) automatically advances at selected intervals the disc (K) that transports the cigarettes on the disc into burning position. Also an electric fuse (H') tripped by a micro-switch, ignites a cigarette when it comes into burning position.

Air is drawn into the control chamber (L) through a Seitz filter (M).

Flowmeters are located at (N) and (O). These measure the rate of flow of gas through the smoking chamber and control chamber respectively. Thus an estimate of air volume passing through the chambers is possible. At (P) and (Q) are the openings used for determining air pressures in both cages by a manometer. These openings are also used for collection of oxygen and carbon dioxide samples.

Experimental mice are placed in wire baskets on the rack (R). Control animals are placed in wire baskets on the rack (S).

A cotton filter is located at (B) and also at (T). These remove tars from the gas before they are released into the room. A room exhaust fan is located next to the pump (A).

A rubber gasket is located between the cigarette house (G) and smoking chamber (E). Also, a rubber gasket is located between the lid (with handles) and the two chambers of the apparatus (experimental (E) and control (L)).

Screws, like (U) and (V) provide an airtight seal.

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